PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference VUB-013-PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/010926	International filing date *(day/month/year)* 30.09.2004	Priority date *(day/month/year)* 03.10.2003
International Patent Classification (IPC) or national classification and IPC G01N15/02		
Applicant VRIJE UNIVERSITEIT BRUSSEL et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20.07.2005	23.08.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Bravin, M Telephone No. +49 89 2399-2417

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/010926

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-14	as originally filed
Claims, Numbers	
1-33	received on 01.08.2005 with letter of 20.07.2005
Drawings, Sheets	
1/7-7/7	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/010926

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-33
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-33
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-33
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1.
Reference is made to the following documents:

D1: WO 03/008931 A (GASPARINI PAOLO ;HVICHIA GEORGI (US)) 30 January 2003 (2003-01-30)
D2: ANONYMOUS: "Dispersion Analysis. March 1982" IBM TECHNICAL DISCLOSURE BULLETIN, IBM CORP. NEW YORK, US, vol. 24, no. 10, March 1982 (1982-03), pages 5038-5040, XP002087335 ISSN: 0018-8689
D3: WO 01/54784 A (MESOSYSTEMS TECHNOLOGY, INC) 2 August 2001 (2001-08-02)
D4: WO 02/087761 A (DESMET GERT ;BARON GINO (BE); UNIV BRUXELLES (BE)) 7 November 2002 (2002-11-07)
D5: DESMET ET AL: "Shear-flow-based chromatographic separations as an alternative to pressure-driven liquid chromatography" JOURNAL OF CHROMATOGRAPHY A, vol. 948, 2002, page 19-34, XP002271305
D6: US 2002/038787 A1 (HURWITZ MARK F ET AL) 4 April 2002 (2002-04-04)

2.
Claim 1 meets the requirements of Art. 33(1)(2)(3) PCT in respect of novelty and inventive step, the reasons being as follows:

The document D1 is regarded as being the closest prior art to the subject-matter of claim 1, and shows a method for separating particles (cells) in a fluid according to size (see abstract, D1), the method comprising the step of transporting a fluid containing said cells across a microprofiled surface carrying a plurality of adjacent regions of different depth which form surface level steps (see [0016] and Fig. 1, D1)

The subject-matter of claim 1 notably differs from this known method in that claim 1 defines that *"the fluid is transported by mechanically moving a flat surface across the profiled surface"*, thereby *"allowing the separation of said particles by means of the*

backflow of excluded particles, said backflow generated by moving said first surface past said profiled surface".

In D1, the profiled surface is covered with a plate (10, Fig. 1, D1) which, together with the profiled surface, forms a flow channel (see [0019], D1). Although in some embodiments said cover can be moved by mechanical means associated to it, the motion envisioned does not occur across the profiled surface but, generally speaking, perpendicularly to said surface, its purpose moreover not for generating a backflow but for adjusting the height of the channel (see [0020], D1).

The subject matter of claim 1 is thus new over D1 (Article 33(2) PCT).

The problem to be solved by the present invention may be regarded as defining an alternative method for generating particle flow across a similar microprofiled structure as known from D1, for the purpose of size-separation.

The solution to this problem as proposed in claim 1 is not suggested by the prior art documents cited in the International search report:

Document D2 discloses a conventional, macroscale grinding gage for dispersing solid particles in a solution by mechanically moving a flat tool over a channel. No backflow is involved.

Document D3 defines a similar structure as in D1 for size-separating aerosols in a flow (see Fig. 5a, D3). The profiled surface is covered by a fixed plate and no alternative is envisioned.

Documents D4 and D5 both disclose the concept of generating shear-driven flow in a microdevice by means of a plate moving accross a micromachined channel. The context is however different since D4 and D5 are concerned respectively with affinity reactions and chromatography, where separation of the particles (molecules) is not directly based on size. As such, D4 and D5 do not provide any incentive to use a shear-driven flow for moving cells in the context of D1.

Document D6 discloses size-separation of particles using a shear flow. The separation, however, occurs through a membrane over which the flow takes place and not through a profiled surface according to claim 1. No alternative is envisioned.

The subject matter of claim 1 is thus inventive (Art.33(3) PCT).

3.
Claim 18 defines a device comprising features corresponding to those defined in claim 1 and meets the requirements of Art. 33(2)(3) PCT for similar reasons as given above for claim 1, document D1 being regarded as closest prior art.

4.
Claims 2-17, resp. claims 19-33, are dependent on claim 1, resp. claim 18, and as such also meet the requirements of the PCT with respect to novelty and inventive step.

Re Item VIII
Certain observations on the international application

1.
The description on p. 9, lines 17-18 discloses a range of step sizes between 2 nanometer and 200 micrometer and thus not in conformity with claims 1 and 18 (Art. 6 PCT). The lower limit of 2 nanometer is regarded as printing mistake in view of (i) the technical effect to be achieved in the present context (i.e. the separation of super-micrometer particles using recirculating flow, see p. 2, lines 3-17) and (ii) the current technical impossibility to precisely machine level steps as envisioned at the nanometer level.

2.
Claim 30 is unclear (Art. 6 PCT) as the feature "the channel inlet" in claim 30 has no antecedent basis in any of claims 18 to 28, on which claim 30 depends.

Claims (retyped)

1. A method for separating particles in a fluid according to size comprising the steps of
a) transporting a fluid containing said particles across a profiled surface carrying at least two adjacent regions of different depth which form a surface level step, wherein
- the fluid is transported by mechanically moving a flat first surface across the profiled surface,
- the adjacent regions of different depth are arranged such that the depth of the regions decreases in the net direction of a forward displacement of the first surface,
- the depth of the regions is between 2 and 200 micrometers,
- force is applied such that one surface is pushed towards the other surface, and
b) allowing the separation of said particles by means of the backflow of excluded particles , said backflow generated by moving said first surface past said profiled surface.

2. A method according to claim 1, wherein where the first surface overlaps with the profiled surface, the first surface lies flat and parallel to the portions of the profiled surface without regions of different depth.

3. A method according to claims 1 and 2 wherein where the first surface overlaps with the profiled surface, at least the region(s) of different depth overlap with the first surface.

4. A method according to any of claims 1 to 3 further comprising the step of collecting the particles from one or more adjacent regions of different depth.

5. A method according to any of claims 1 to 4, wherein the widths of two or more regions adjacent to the surface level step are different.

6. A method according to any of claims 1 to 5 wherein the regions of different depth are micro machined.

7. A method according to any of claims 1 to 6, wherein the first surface moves in an intermittent mode.

8. A method according to any of claims 1 to 7, wherein the first surface moves alternately forwards and backwards, each movement having a duration and a velocity selected such that the net displacement is in the forward direction.

9. A method according to any of claims 1 to 8, wherein one or more said regions of different depth regions each comprise an opening into a chamber.

10. A method according to any of claims 1 to 9, wherein said particles are non-covalently bound to said first surface before they reach said surface level step.

11. A method according to any of claims 1 to 10, wherein a selective force field is applied to selectively and temporarily direct at least one fraction of the particles towards a predetermined surface during a given period.

12. A method according to any of claims 1 to 11, wherein a side-outlet channel is provided near at least one side of said surface level step.

13. A method according to any of claims 1 to 12, wherein the particles are collected after the separation by applying a second flow parallel to said surface level step.

14. A method according to any of claims 1 to 13, wherein said fluid substances are continuously fed at a channel inlet and are continuously withdrawn from one or more outlet channels.

15. A method according to any of claims 1 to 14 further comprising, the step of collecting particles at said outlet channel(s).

16. A method according to any of claims 1 to 15, wherein the direction of said surface level step and the mean direction of the flow cross at an angle between 1° and 90°.

17. A method according to any of claims 1 to 16, wherein said fluid substances are fed at a limited section of the channel inlet only.

18. A device for separating particles in a fluid according to size comprising:

- a profiled surface carrying at least two adjacent regions of different depth which form a surface level step, in which the depth of the regions is between 2 and 200 micrometers,
- a flat first surface that is capable of mechanically moving across the profiled surface, and

- a means for mechanically moving said first surface over the profiled surface,

wherein the adjacent regions of different depth are arranged such that the depth of the surface level steps decreases in the net direction of the forward displacement of the first surface.

19. A device according to claim 18 wherein where the first surface overlaps with the profiled surface the first surface lies substantially flat and parallel to the portions of the profiled surface without regions of different depth.

20. A device according to claims 18 and 19 wherein at least the region(s) of different depth of the profiled surface overlap with the first surface

21. A device according to any of claims 18 to 20 further comprising a means to apply a pressure to at least one surface.

22. A device according to any of claims 18 to 21, wherein the widths of two or more regions of different depth adjacent to the surface level step are different.

23. A device according to any of claims 18 to 22 wherein the regions of different depth are micro-machined.

24. A device according to any of claims 18 to 23, wherein the first surface is capable of moving in an intermittent mode.

25. A device according to any of claims 18 to 24, wherein the first surface is capable of moving alternately forwards and backwards, each movement having a duration and a velocity selected such that the net displacement is in the forward direction.

26. A device according to any of claims 18 to 25, wherein one or more said regions of different depth regions each comprise an opening into a chamber.

27. A device according to any of claims 18 to 26, wherein a side-outlet channel is provided near at least one side of said surface level step.

28. A device according to any of claims 18 to 27, further comprising a means to apply a second flow parallel to said surface level step.

29. A device according to any of claims 18 to 28, further comprising an inlet channel and one or more outlet channels.

30. A device according to any of claims 18 to 29 further comprising means to continuously feed said fluid to the channel inlet, and withdraw a fluid from one or more outlet channels.

31. A device according to any of claims 19 to 30, wherein the direction of said surface level step and the mean direction of the forward displacement of the first surface cross at an angle between 1° and 90°.

32. A device according to any of claims 19 to 31 wherein the movement of the first surface past the profiled surface generates at least one recirculating flow.

33. Use of a device according to claims 19 and 32 for size-separating particles in a fluid.